Title: Cleaved PINK1 induces neuronal plasticity through PKA mediated BDNF functional regulation

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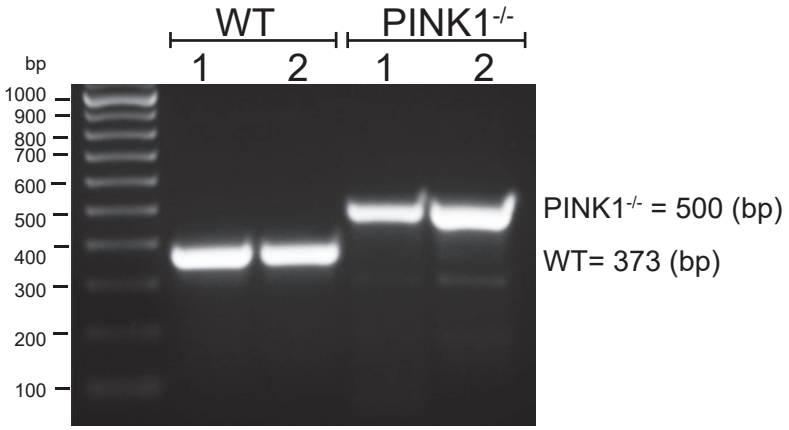
Supplementary figures

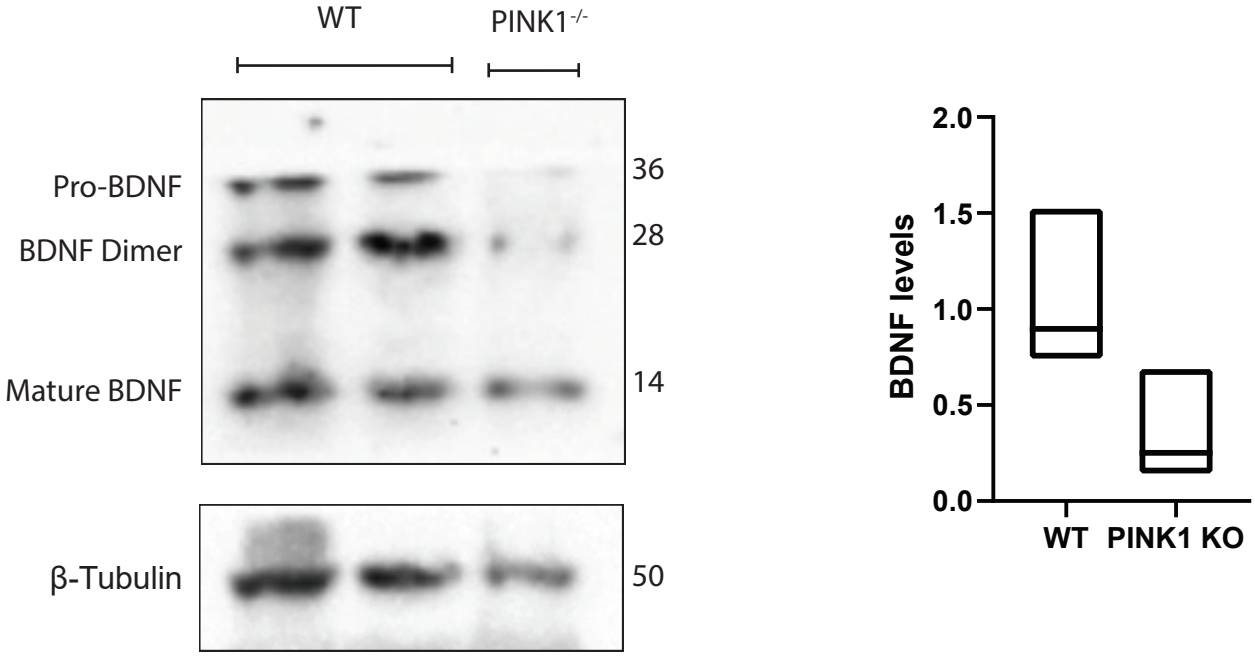
Supplemetary figure 1

1A



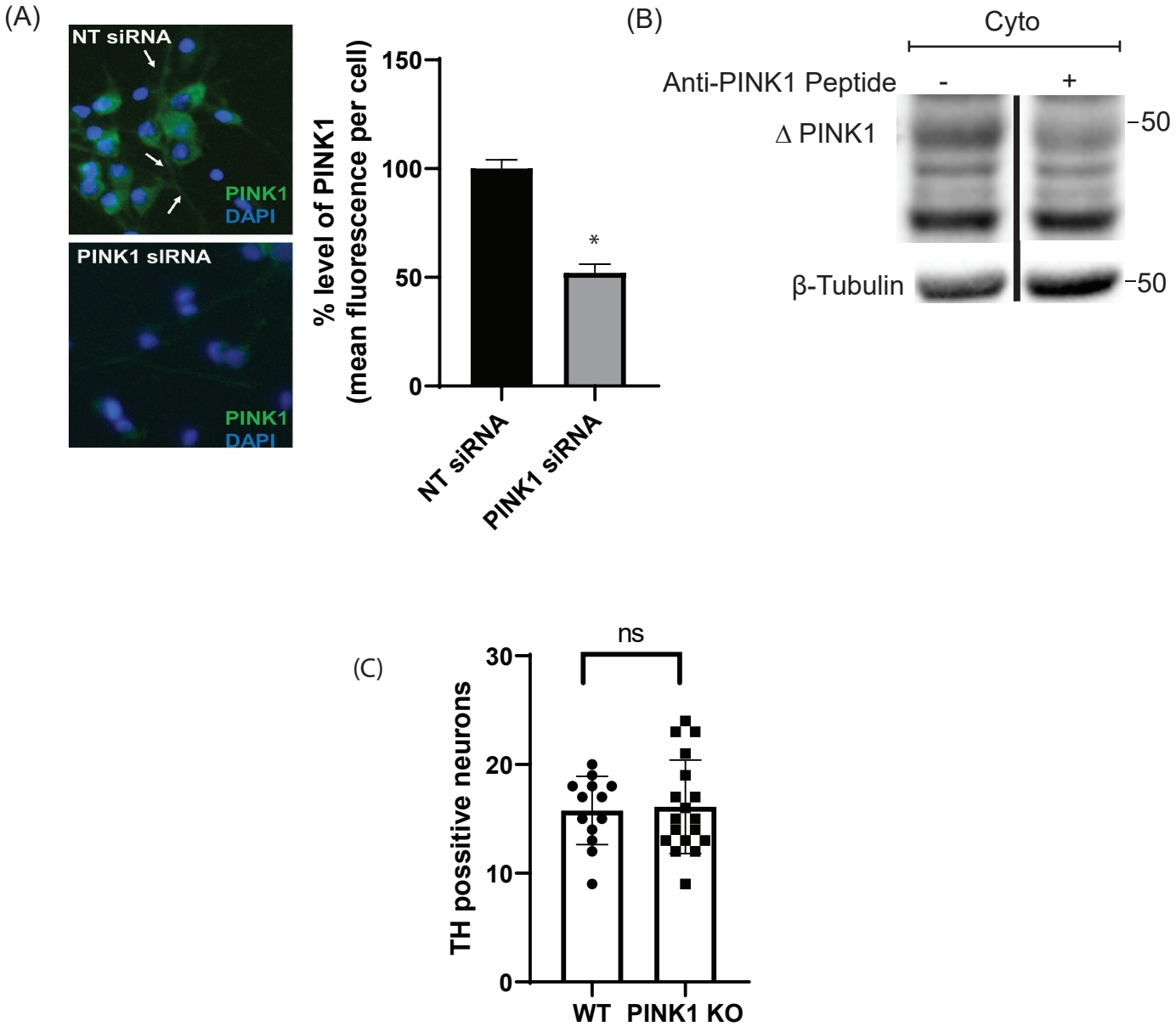
1A: RT-PCR data showing wt Pink1 allele (373bp) in the first and second column; the third and fourth column shows the PINK1 mutant allele (500bp) in homozygous PINK1$^{-/-}$ mouse.

1B



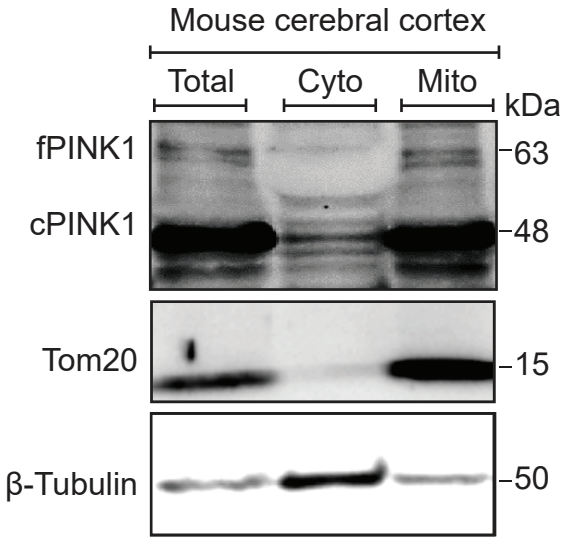
1B: Representative Western blotting for the indicated proteins in lysates extracted 10 month old WT and PINK1KO mouse cerebral cortex. Densitometry analysis of WT and PINK1 KO BDNF levels. Values were normalized to β-Tubulin levels. N=3 (3 mouse per experimental group).

Supplemetary figure 2



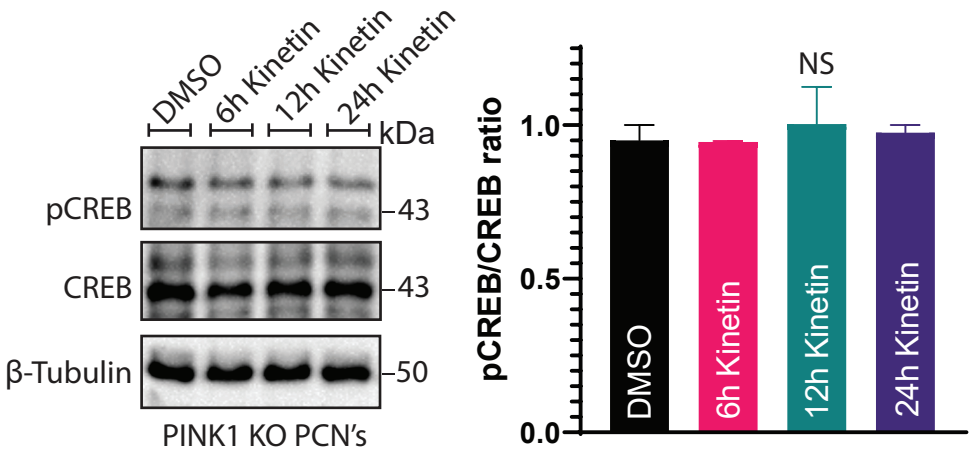
A, Representative immunofluorescence (IF) images of mouse PCNs transfected with a non-targeted siRNA control (NTsiRNA) or with a pre-validated mouse PINK1 siRNA (Life Technologies). 3 days post transfection neurons were immunostained for PINK1 (green) and counterstained with DAPI (blue) to visualize nuclei. Quantification on the right shows IF-based quantification of mean levels (±SEM) of endogenous PINK1 for each condition (*:$p<0.05$ vs. NTsiRNA, 80 neurons per condition, t-test). **B**, Representative Western blotting showing that the immunoreactivity of cleaved PINK1 in the cytosol (cyto) is reduced in the presence of a PINK1 blocking peptide (5 µg peptide :1µg antibody). Discontinuity in the western blot images (shown by a line) indicate that certain lanes have been cropped to maintain visual clarity. The above mentioned data confirms the specificity of anti-PINK1 antibody (; BC100-494; Novus Biologicals, Littleton, CO). **C**, Graphical representation of Tyrosine hydroxylase possitive neurons in substantia nigra of the midbrain section of WT and PINK1 KO 10 month old mice.

Supplemetary figure 3



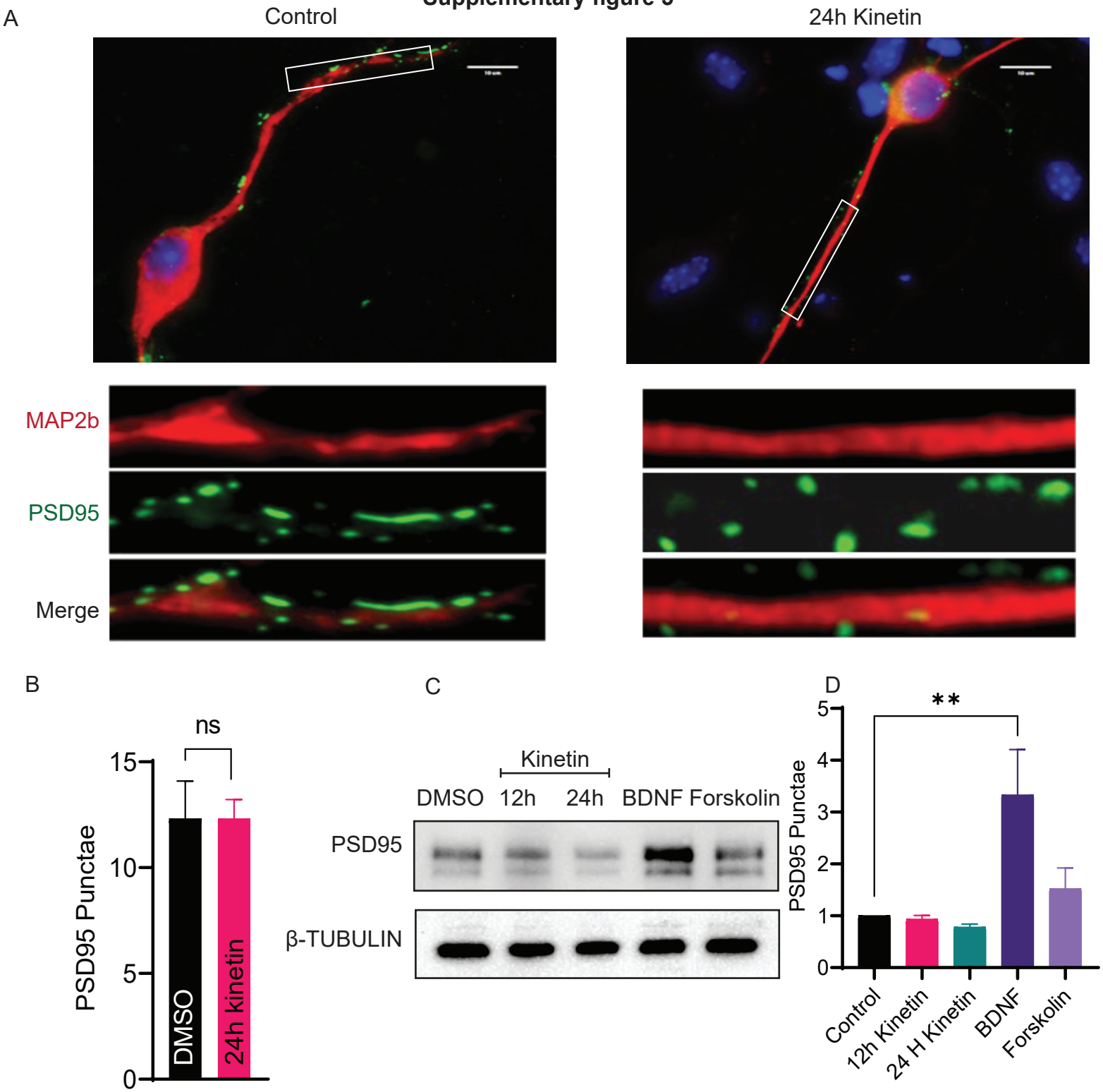
Representative Western blotting for the indicated proteins in lysates extracted after subcellular fractionation of mouse cerebral cortex. Note that 75 μg of total cell lysate, cytosolic and mitochondrial protein was loaded into the gel, which accounts for 12.45%, 1.93%, and 24.79% of the total, cytosolic and mitochondrial lysate, respectively. Densitometry with correction for the relative fraction of total mitochondrial and cytosolic proteins loaded on the gel reveals that 75.48% of the 48kDa cPINK1 is localized to the cytosolic fraction whereas the smaller remnant of cPINK1 is localized to mitochondria.

Supplemetary figure 4



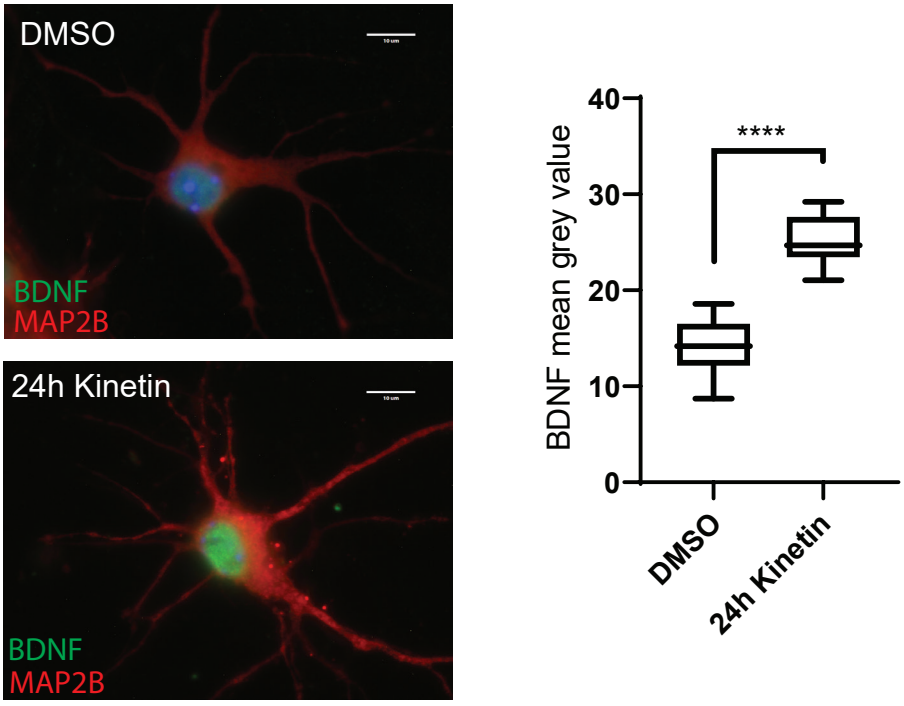
Representative Western blotting for the indicated proteins in cell lysates extracted at different time-points from DMSO, 6h kinetin, 12h kinetin, and 24h kinetin treated PINK1-/- PCNs. Densitometric analysis of Phospho-CREB and CREB expression levels in from DMSO, 6h kinetin, 12h kinetin, and 24h kinetin treated PINK1-/- PCNs. Values were normalized to β-Tubulin.

Supplementary figure 5



A, Representative epifluorescence merged images of PSD-95 (green) immunostaining in PINK1-KO PCNs treated with DMSO (Control) and 24h kinetin. Nucleus is counterstained by DAPI (blue). Magnified images of PSD-95 (green) punctae on dendrites (MAP2b-red). Scale bar represents 10μM. B, Quantification of PSD-95 expression in PINK1-KO PCNs treated with DMSO (Control) and 24h kinetin. An unpaired t-test was used for statistical analysis. Bars denote the average ratio ± SEM and are representative of two independent experiments (N=20; 10 neurons per genotype per experiment). C, Representative Western blot for PSD95 immunoblotting in lysates extracted at differential time-points from DMSO (Control), 4h kinetin, 8h kinetin, 12h kinetin, and 24h kinetin treated PINK1-/- PCNs. F, Densitometry analysis of PSD95 expression levels in DMSO (Control), 4h kinetin, 8h kinetin, 12h kinetin, and 24h kinetin treated PINK1-/- PCNs. Values were normalized to β-Tubulin. Ordinary one-way ANOVA and post-hoc analysis using Dunnett's multiple comparison test were used for statistical analysis. Bars denote the average ratio ± SEM and are representative of two independent experiments.

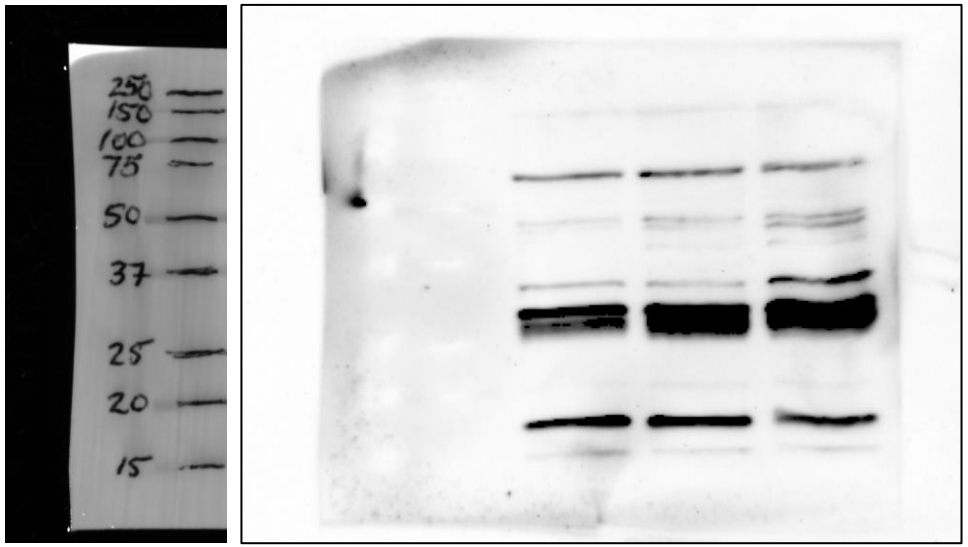
Supplementary figure 6



Representative immunofluorescence (IF) images of WT mouse PCNs treated with Kinetin for 24h. Neurons were immunostained for BDNF (green), MAP2B (Red) and counterstained with DAPI (blue) to visualize nuclei. Quantification on the right shows IF-based quantification of mean levels (±SEM) of endogenous BDNF for each condition (****:p<0.001: 10 Neurons per experimental group, t-test).

Fig1A

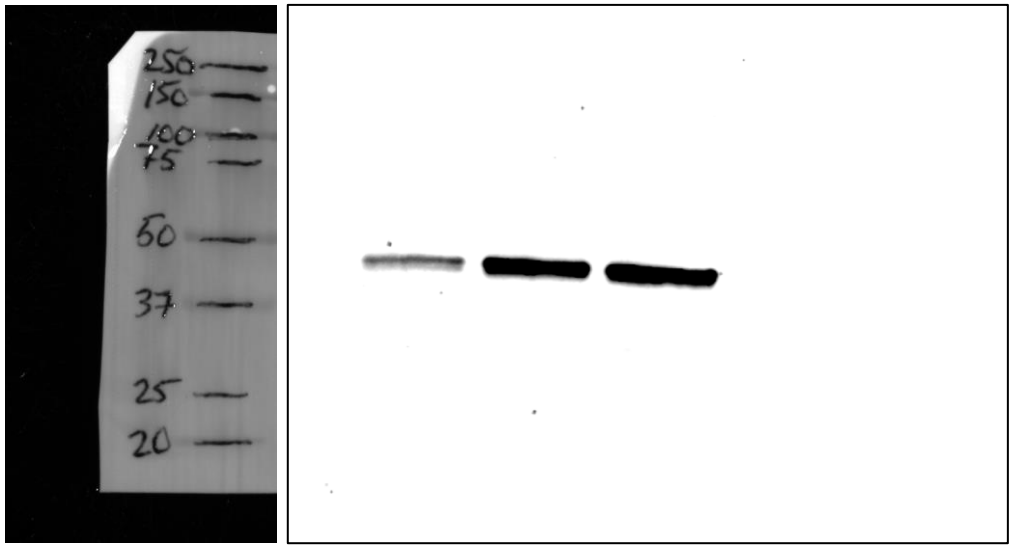
BDNF



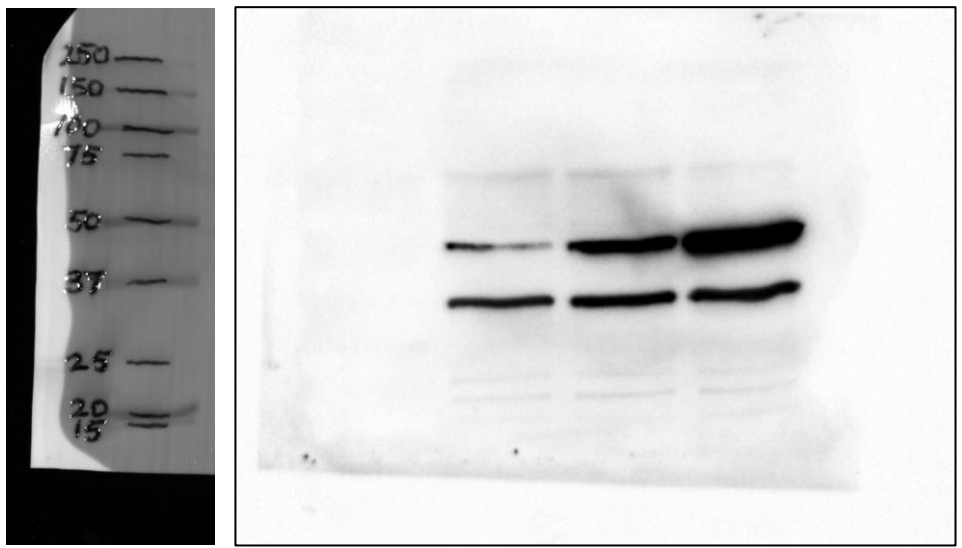
Pro-BDNF
BDNF Dimer

Mature BDNF

P_CREB



PINK1



PSD95

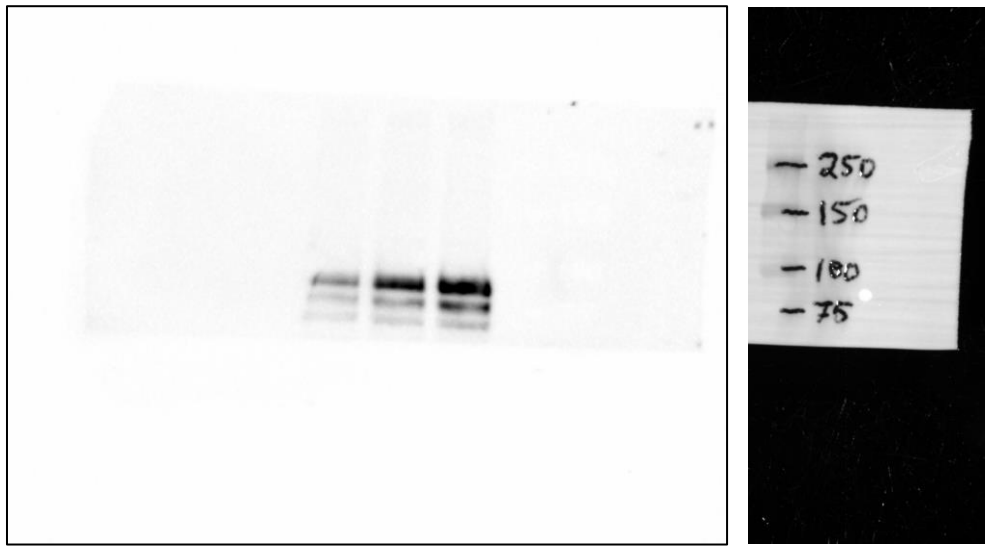
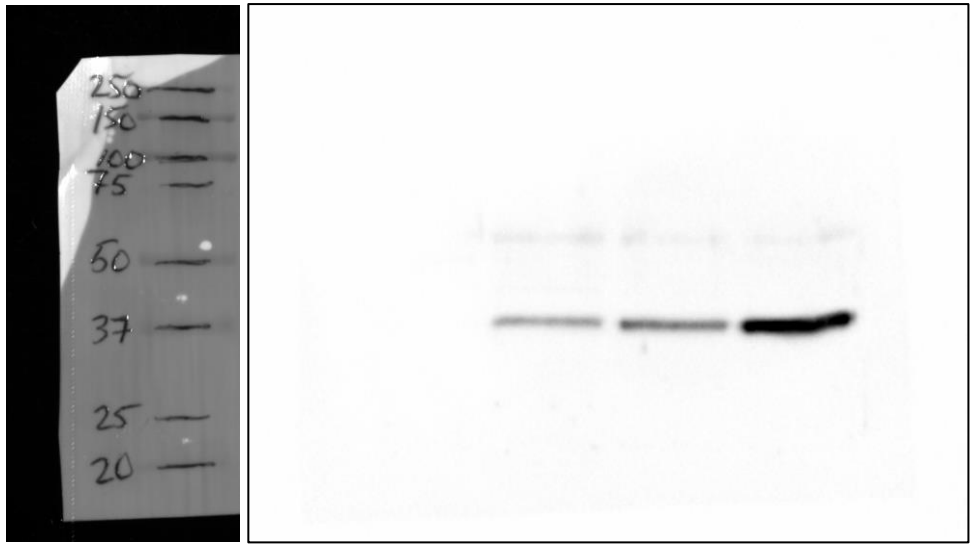


Fig1A

Synaptophysin



β-Tubulin

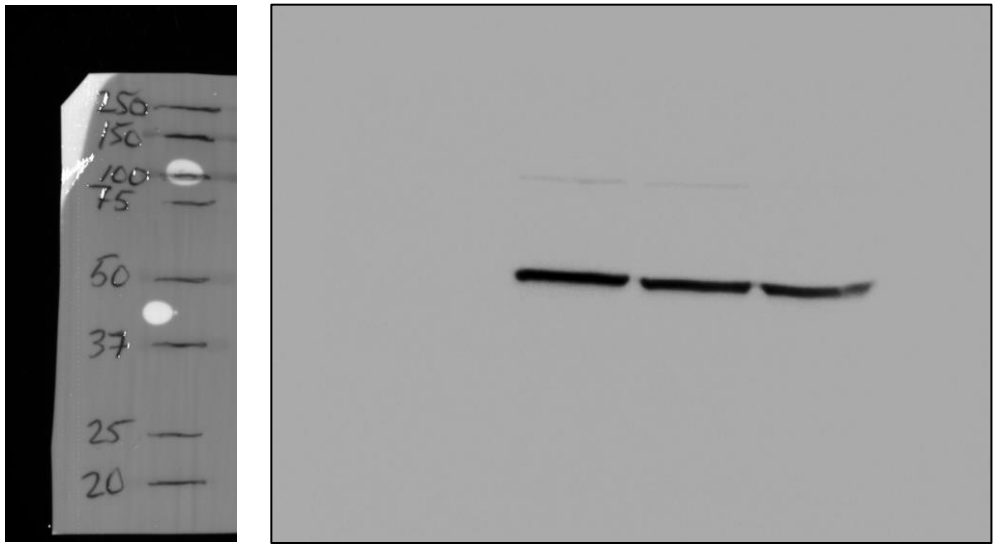
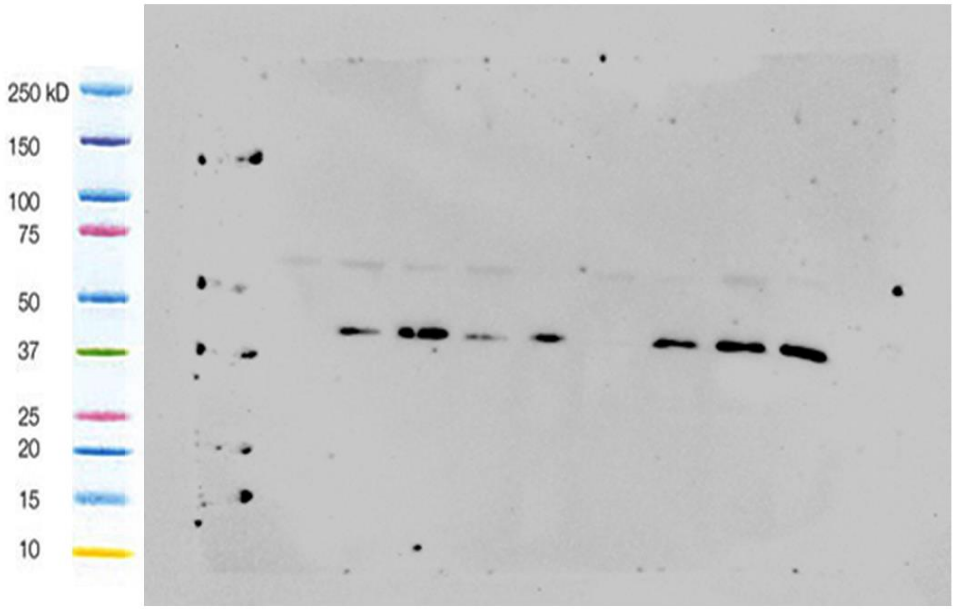


Fig 1D

cPINK1



β-Tubulin

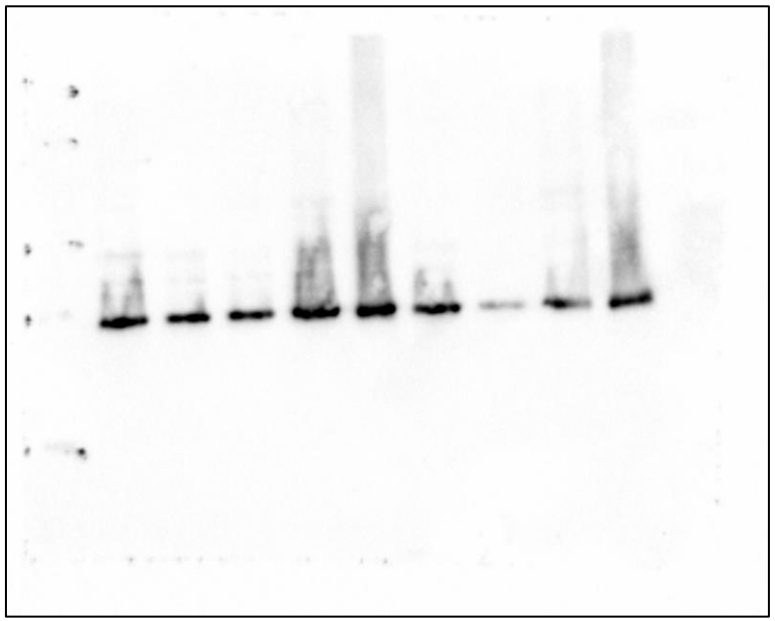
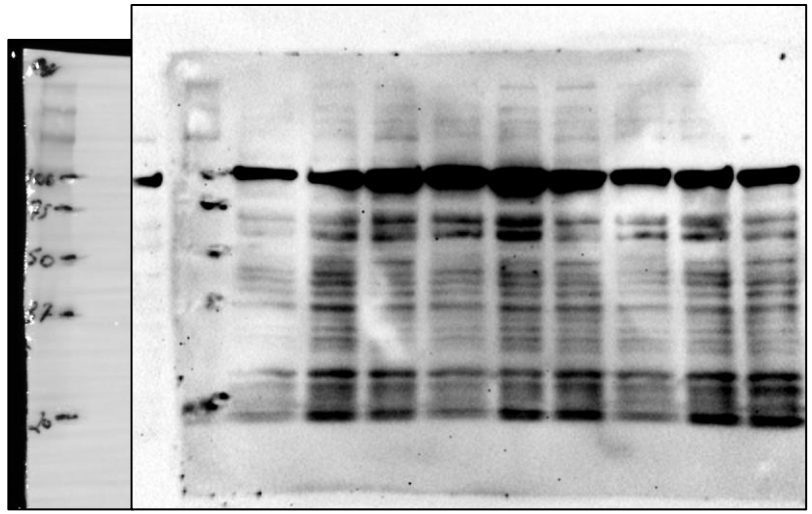


Fig1F

PINK1



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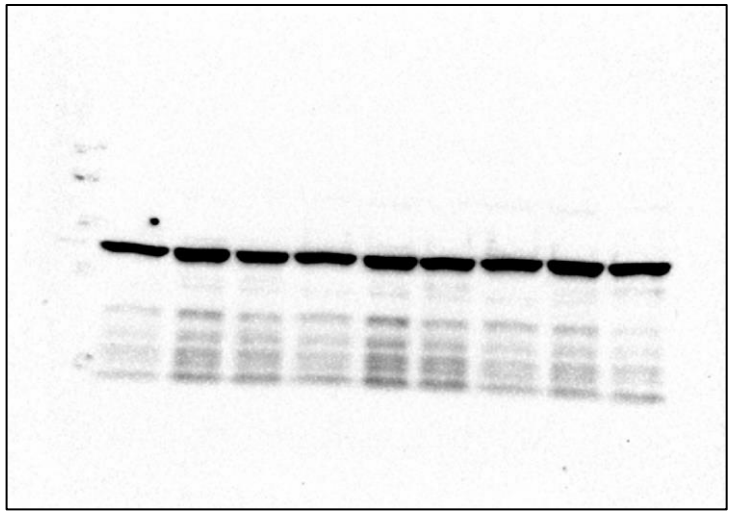
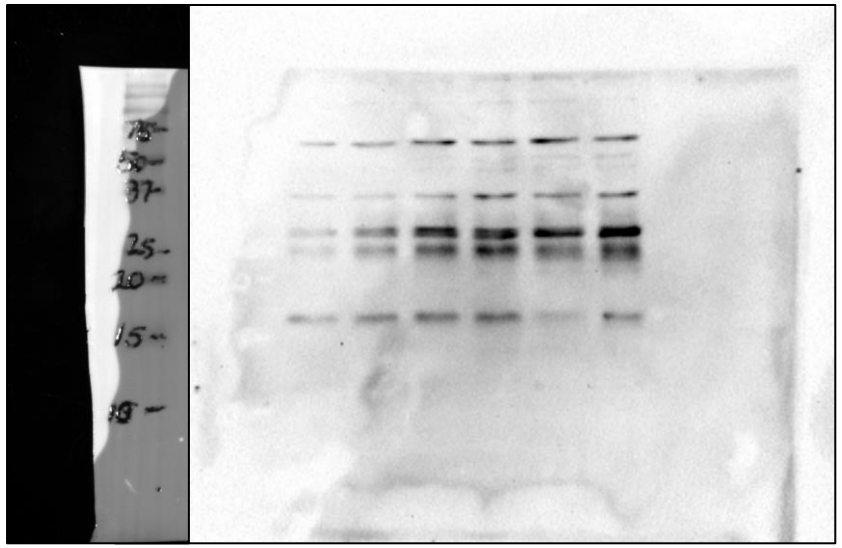


Fig2E

BDNF



β-Tubulin

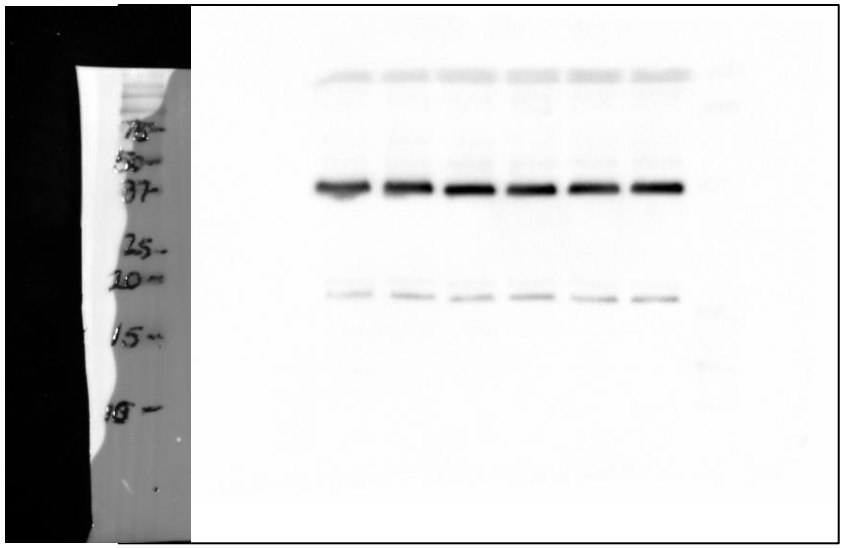
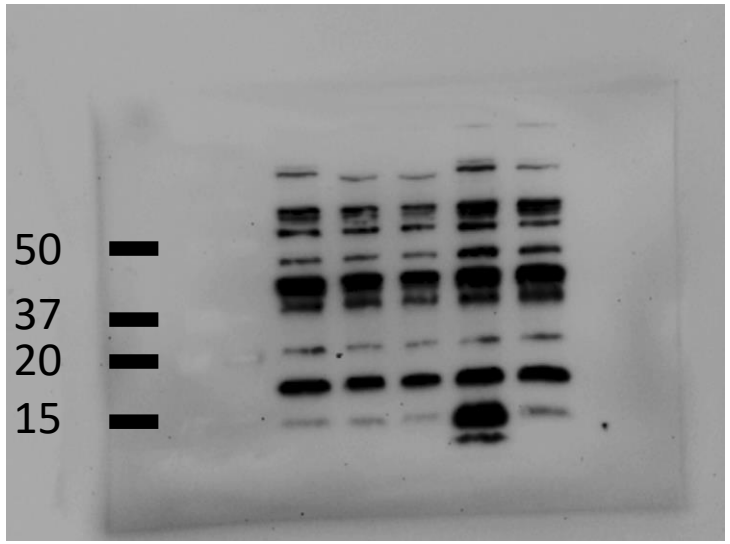


Fig2G

BDNF



β-Tubulin

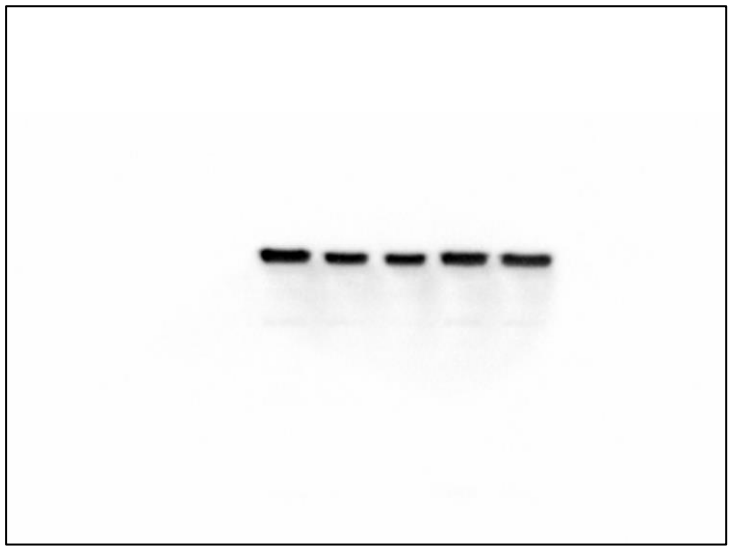
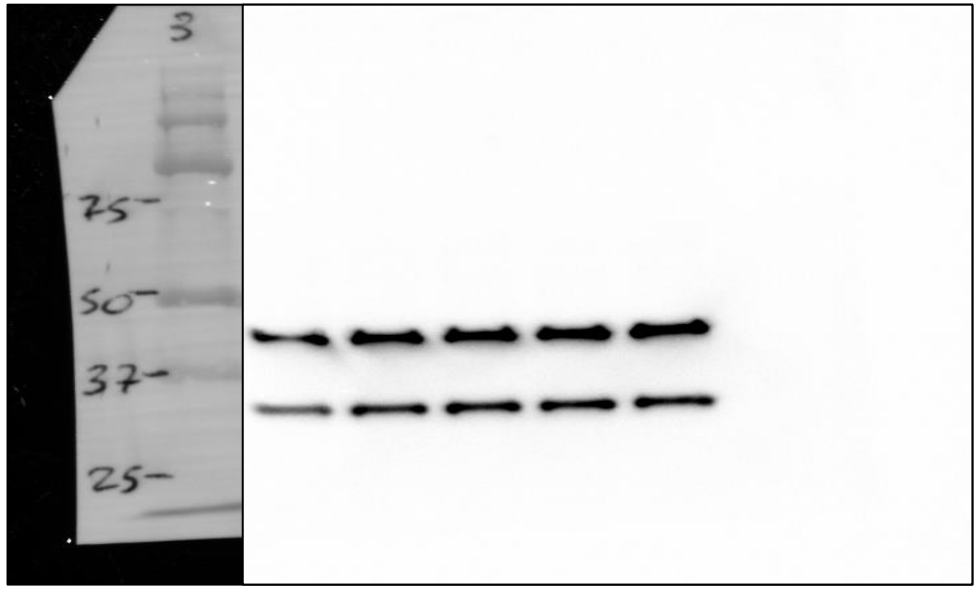


Fig2I

cPINK1



β-Tubulin

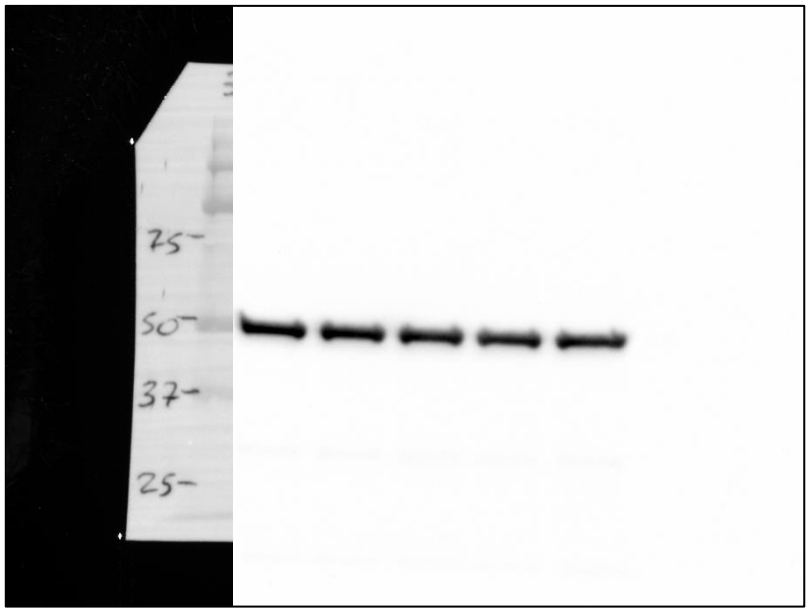
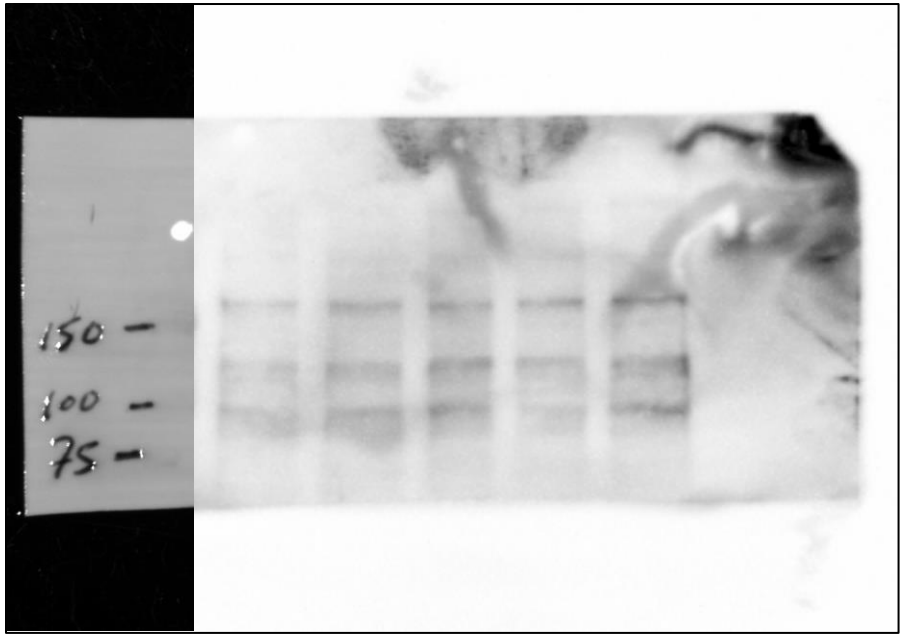


Fig3E

Phospho-TRKβ



β-Tubulin

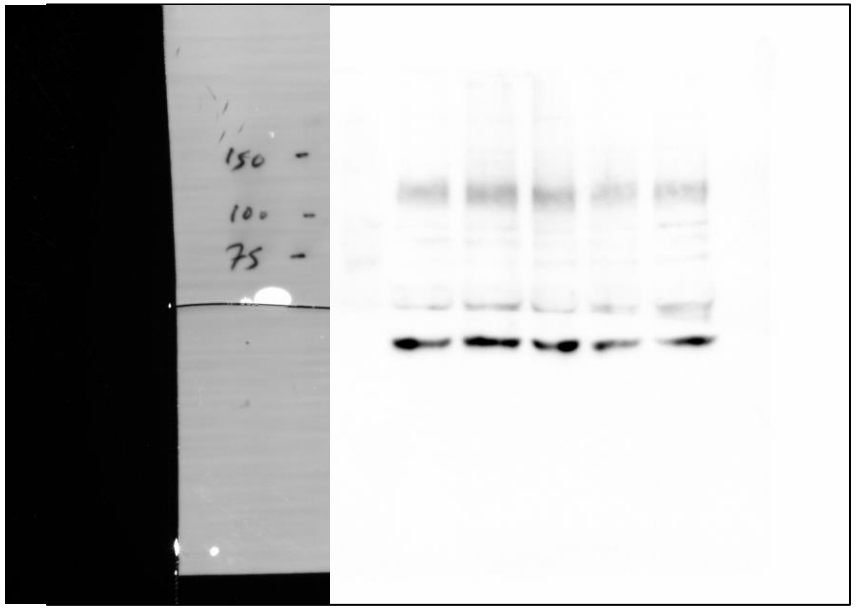
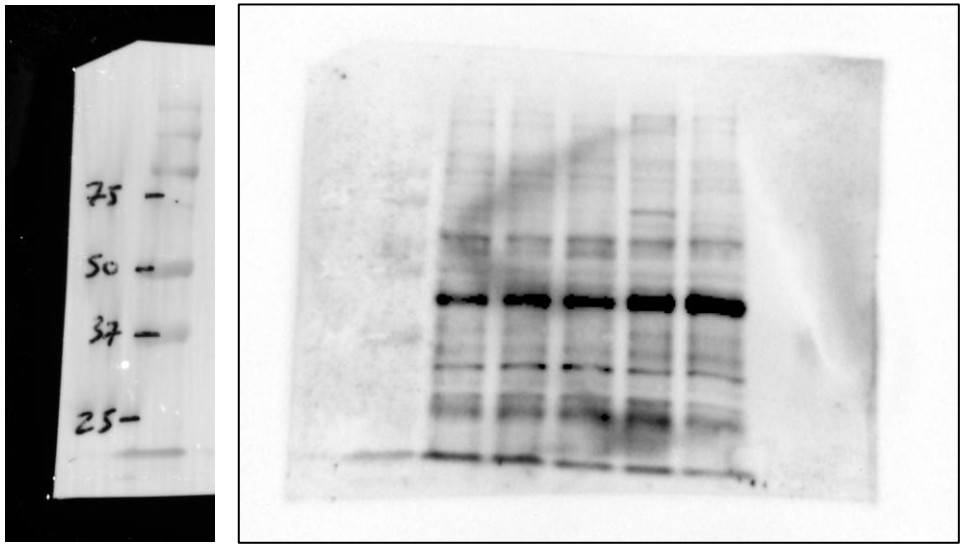
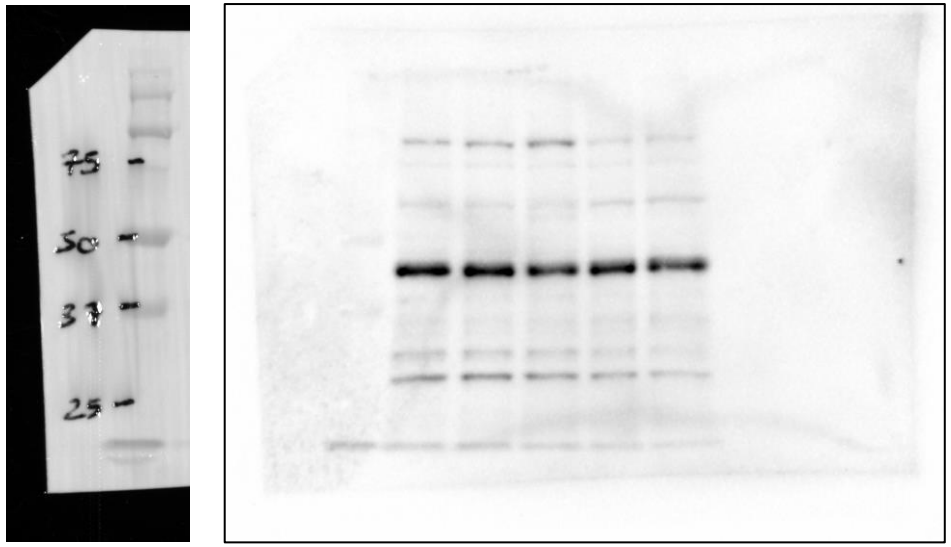


Fig4E

Phospho-CREB



CREB



β-Tubulin

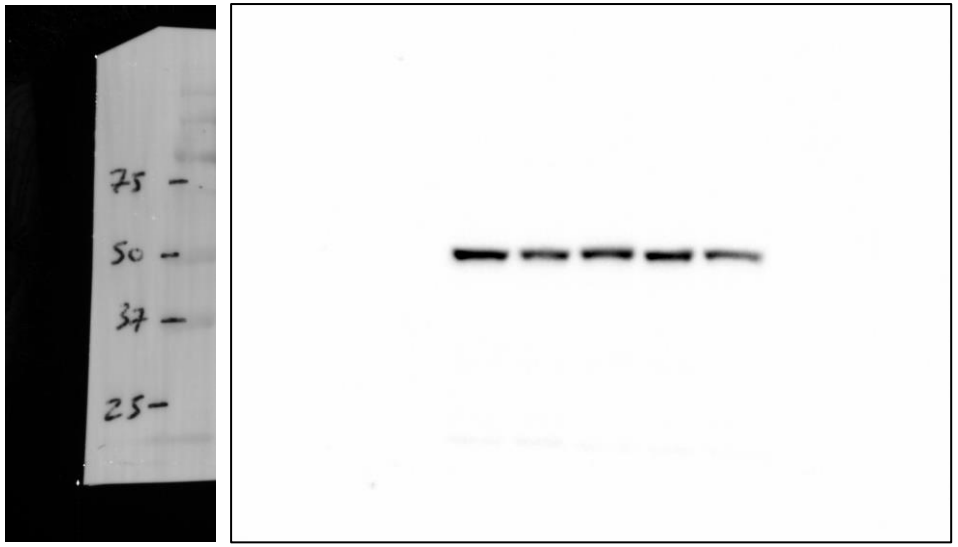
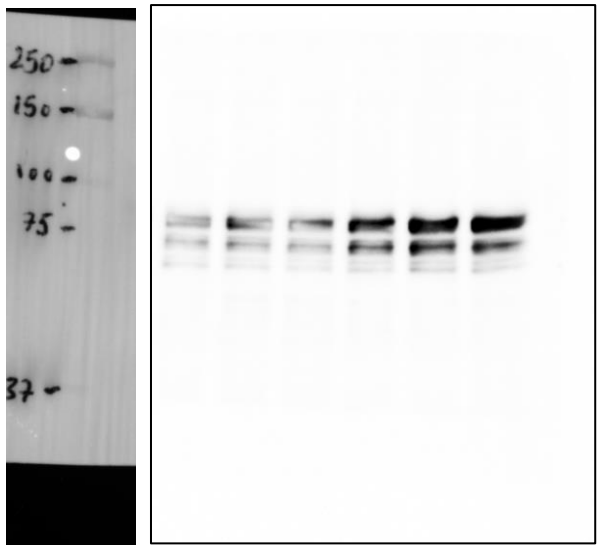
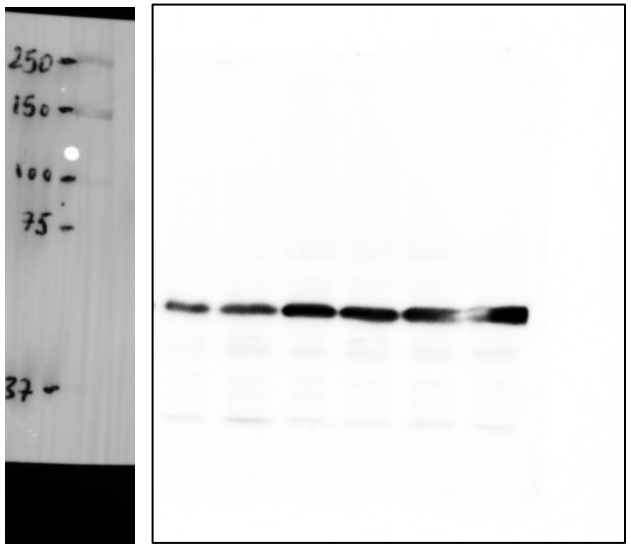


Fig5E

PSD95



Synaptophysin



β-Tubulin

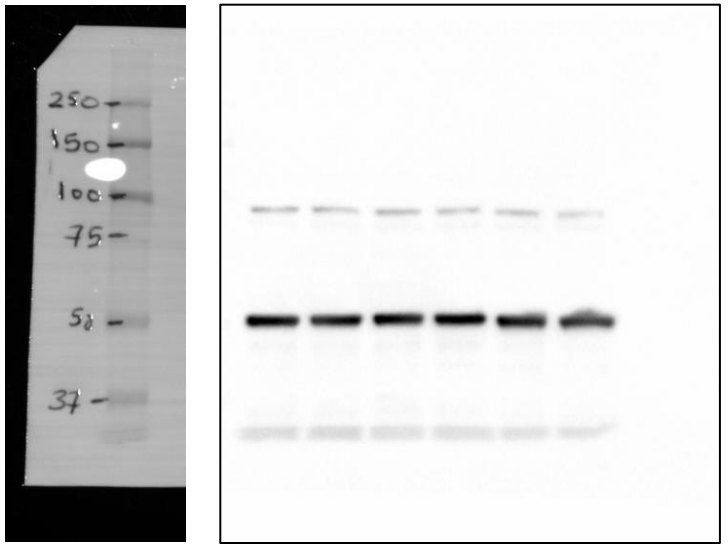
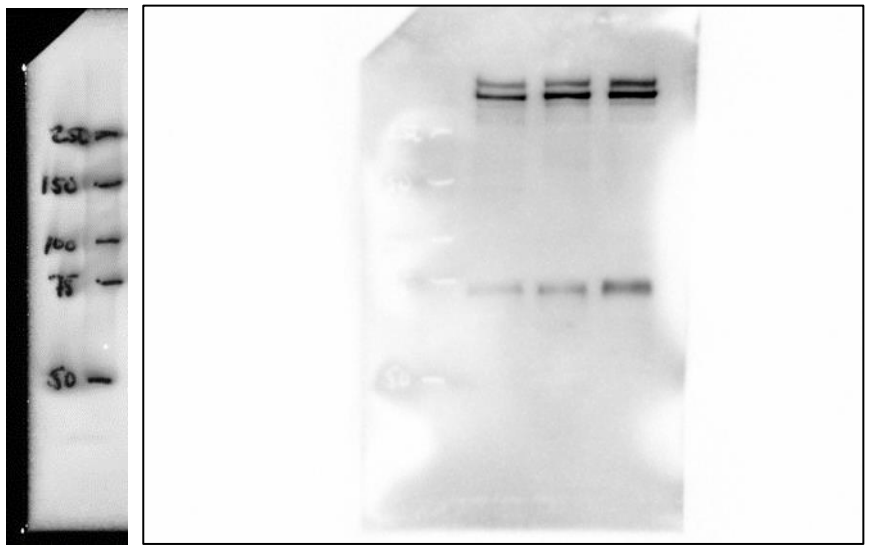
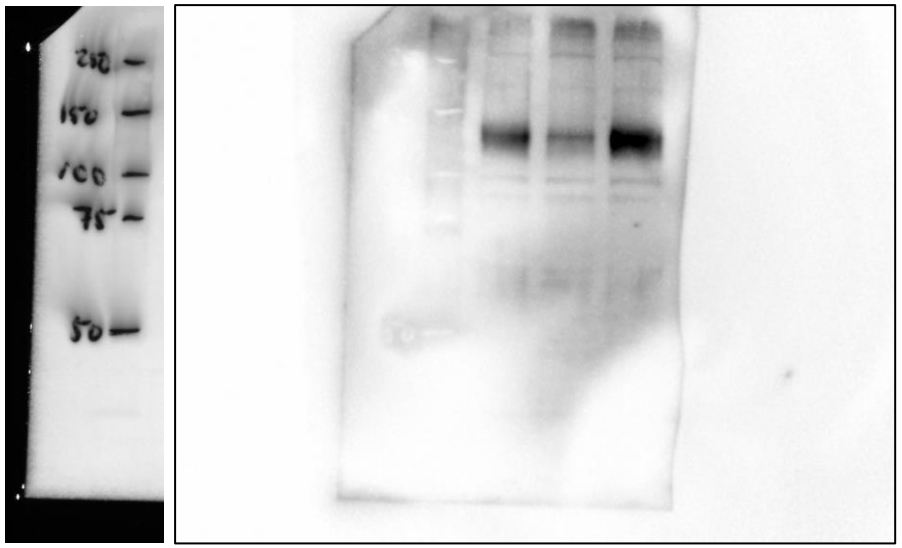


Fig6A

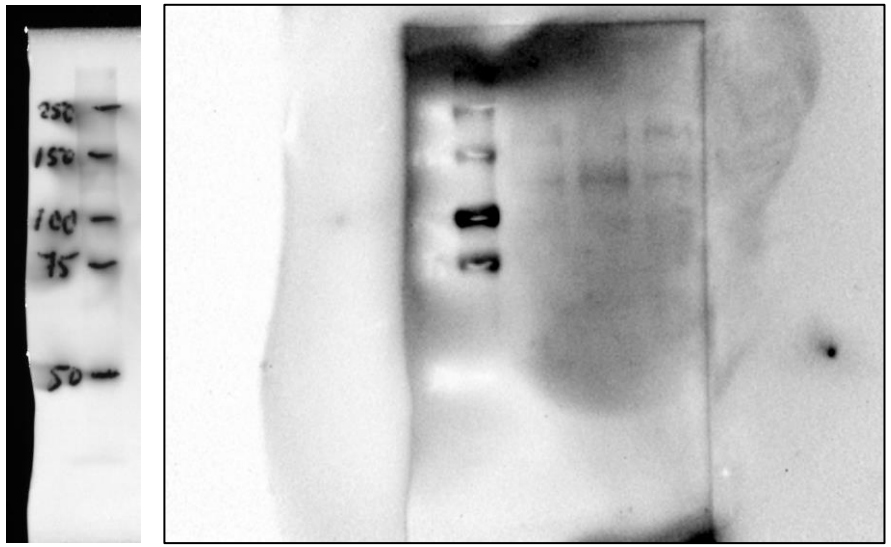
MAP2B



Total TRKβ



Phospho-TRKβ



PSD-95

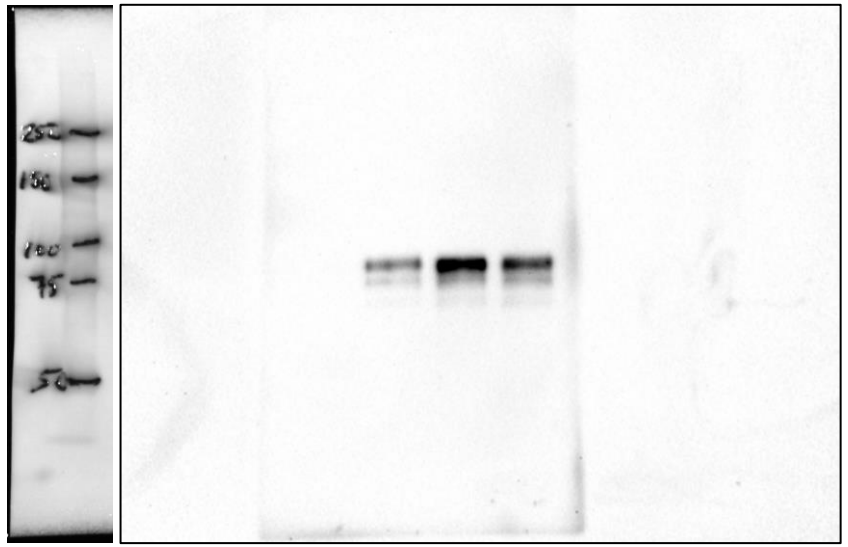
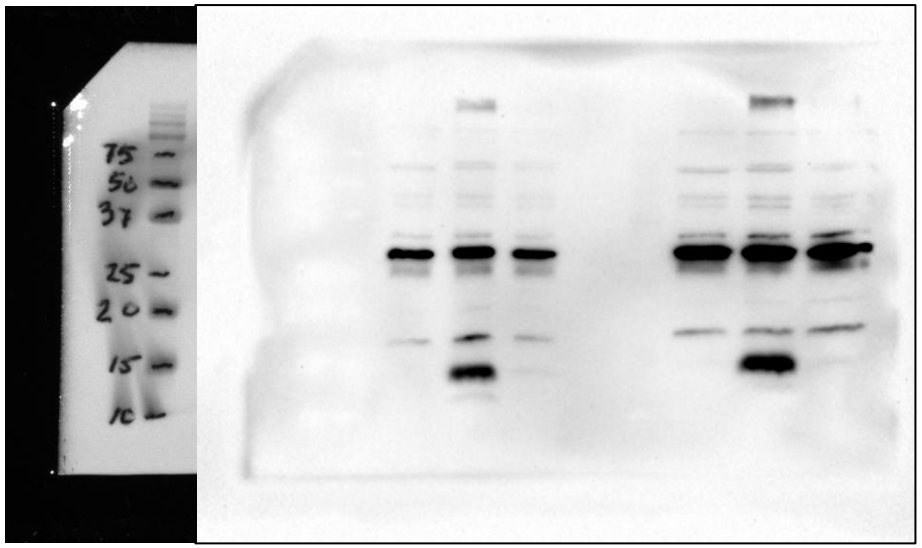
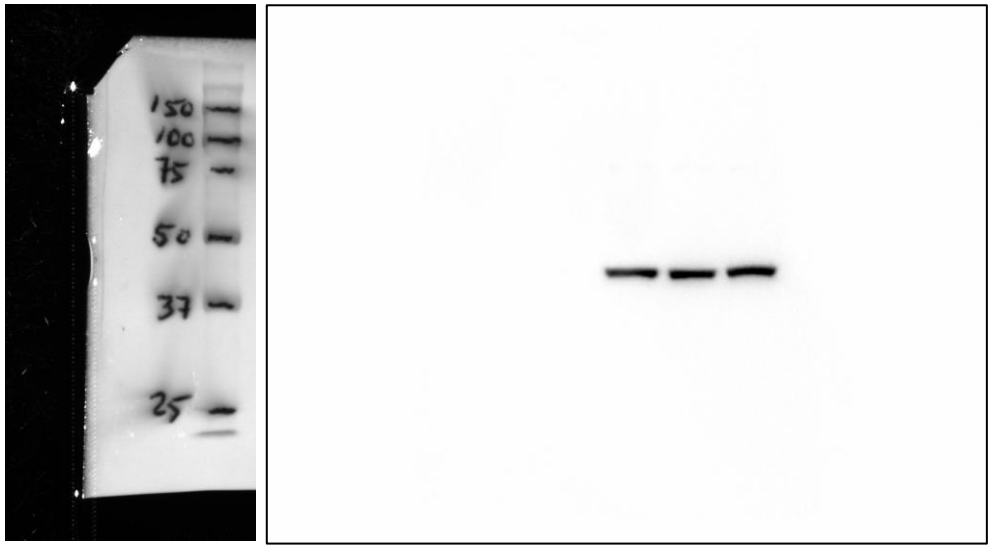


Fig6A

BDNF



β-Tubulin



Phospho-PKA Catalytic (Thr197)

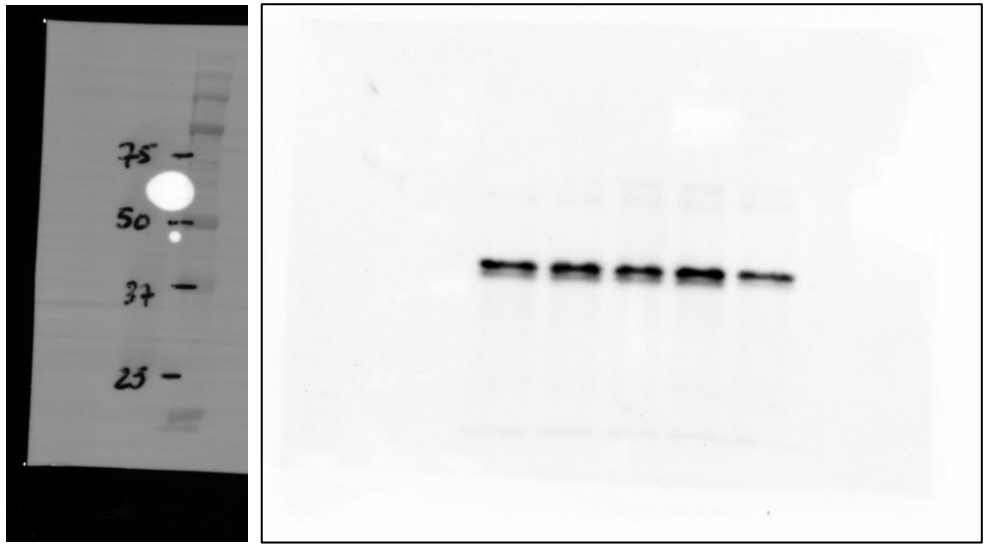
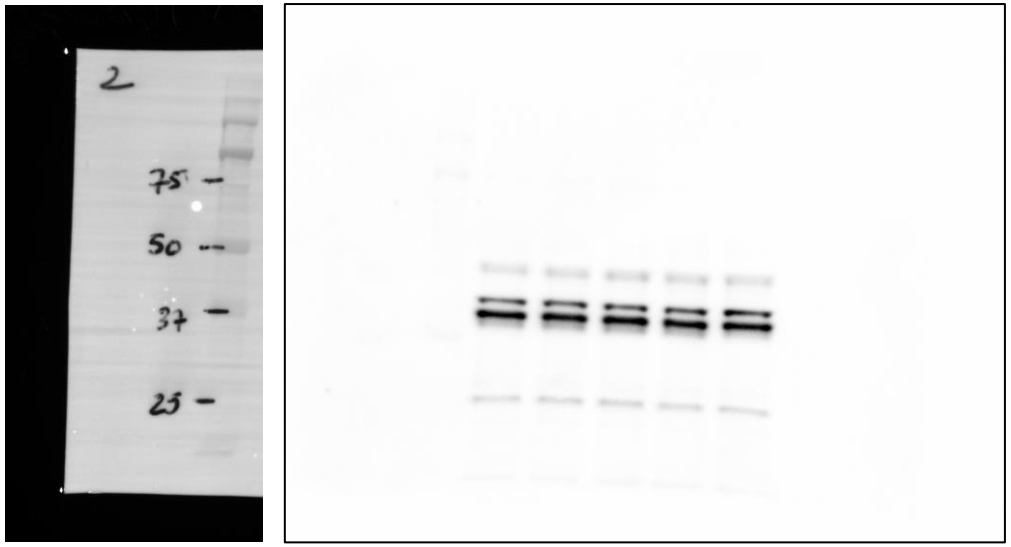


Fig6C

Total-PKA catalytic



β-Tubulin

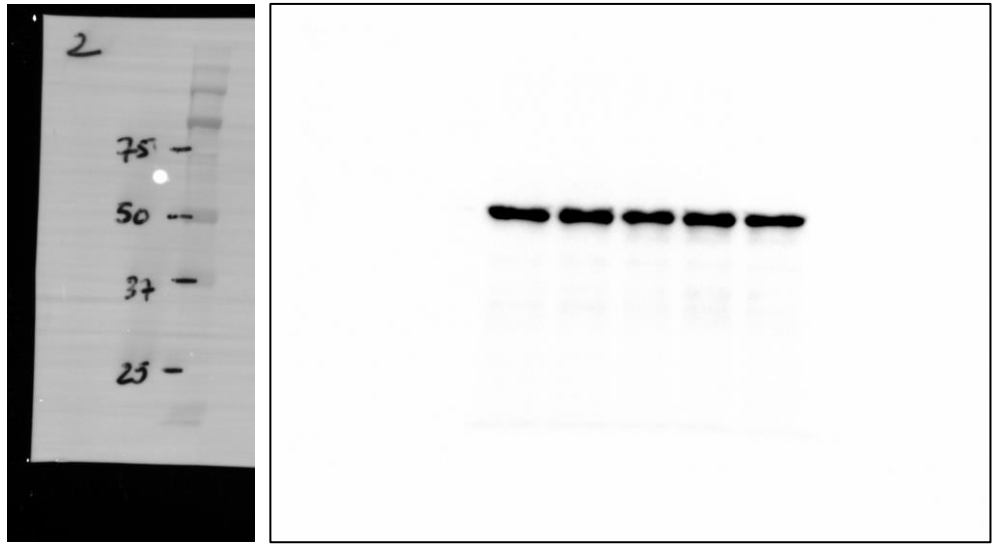
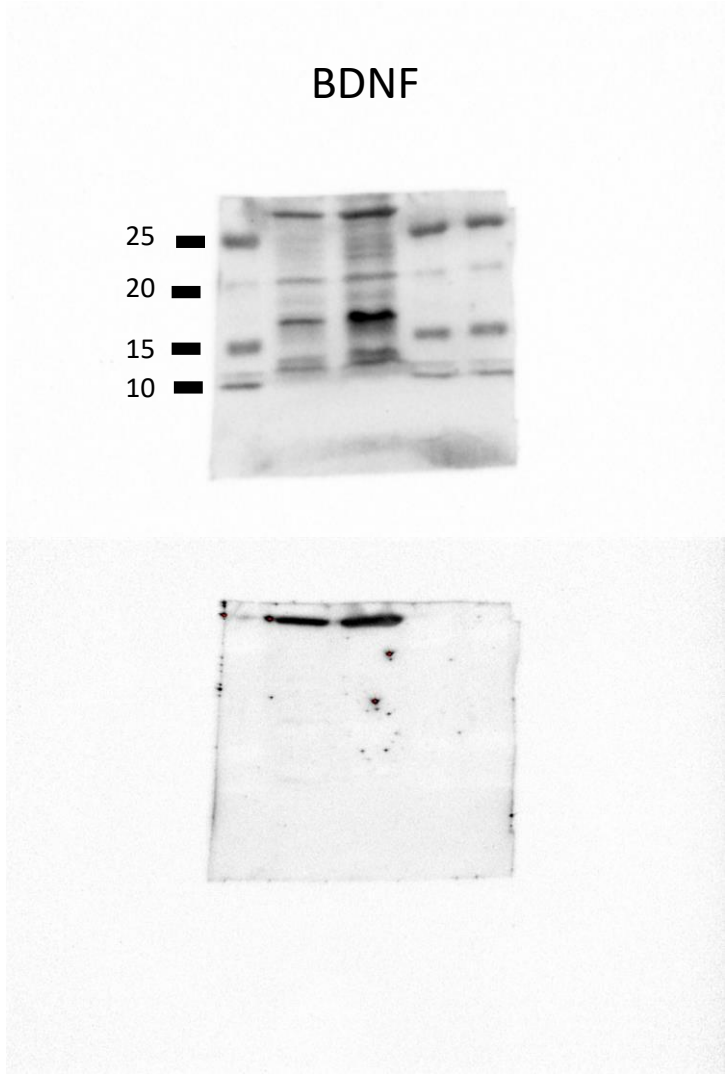


Fig6E



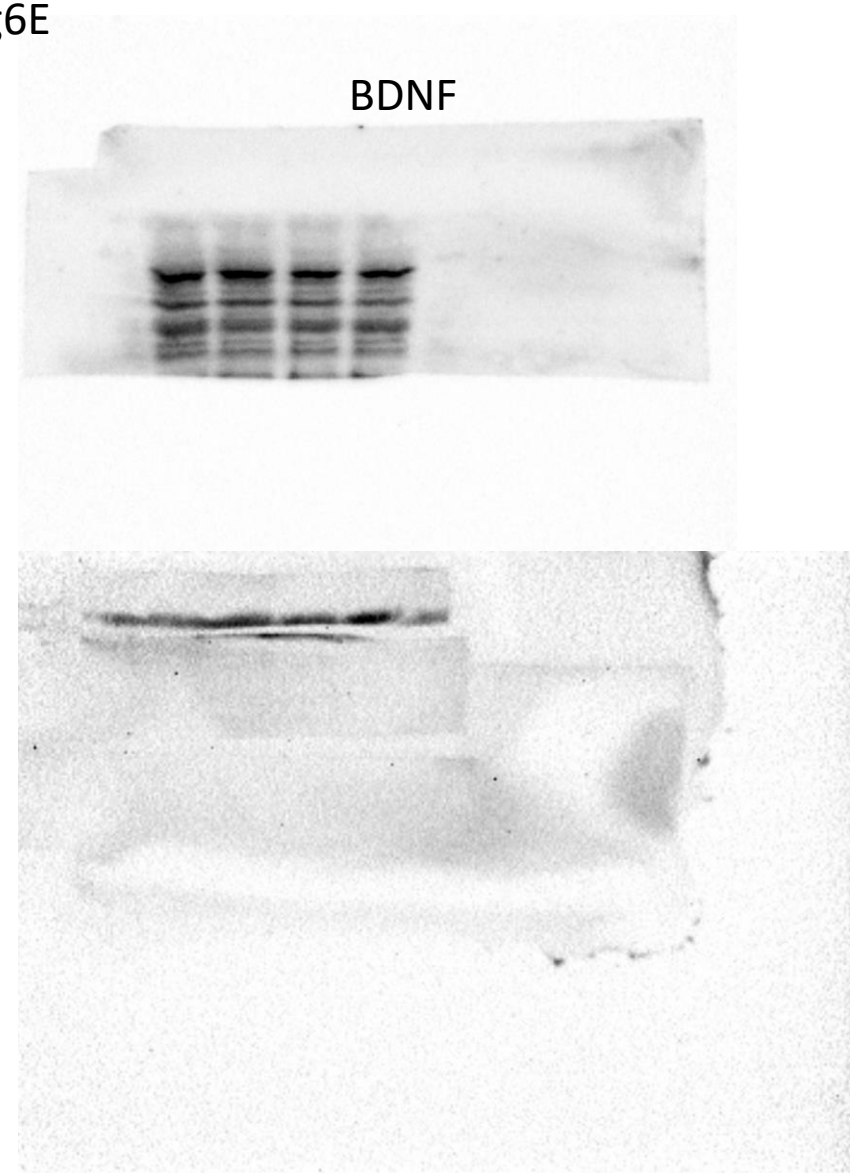
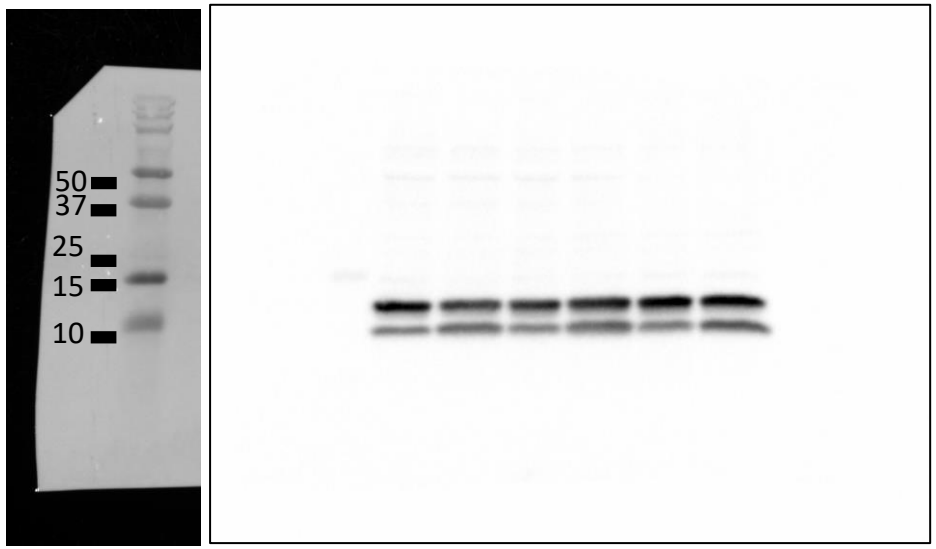
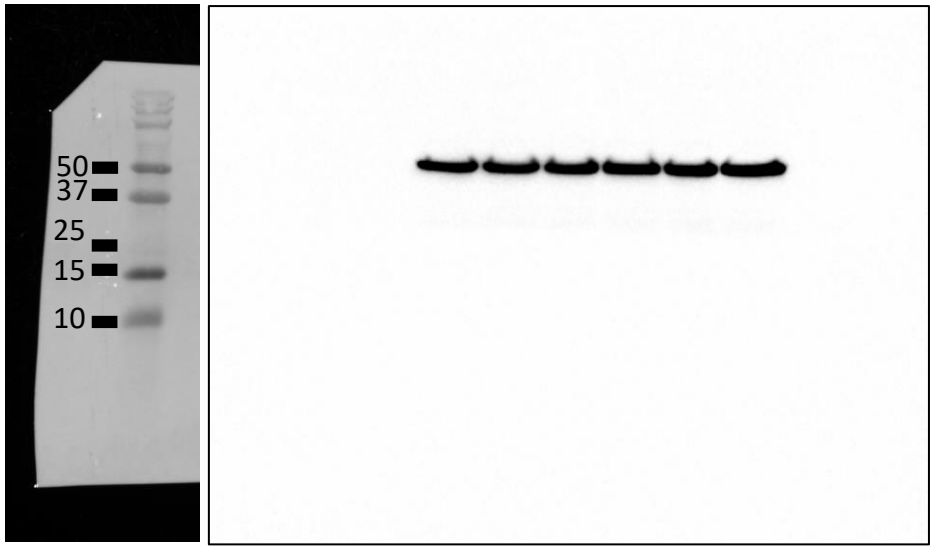


Fig6F

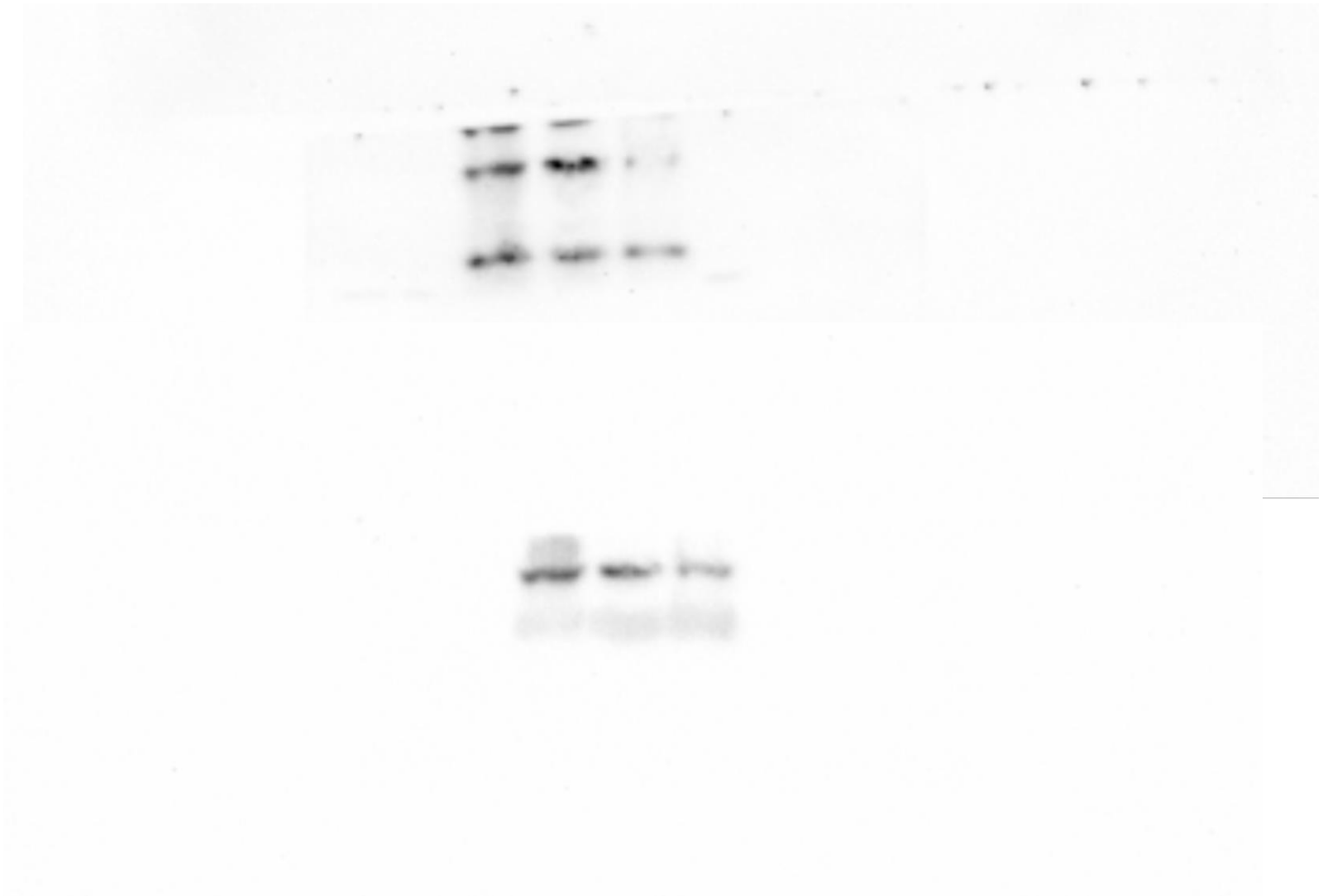
LC-I & II



β-Tubulin

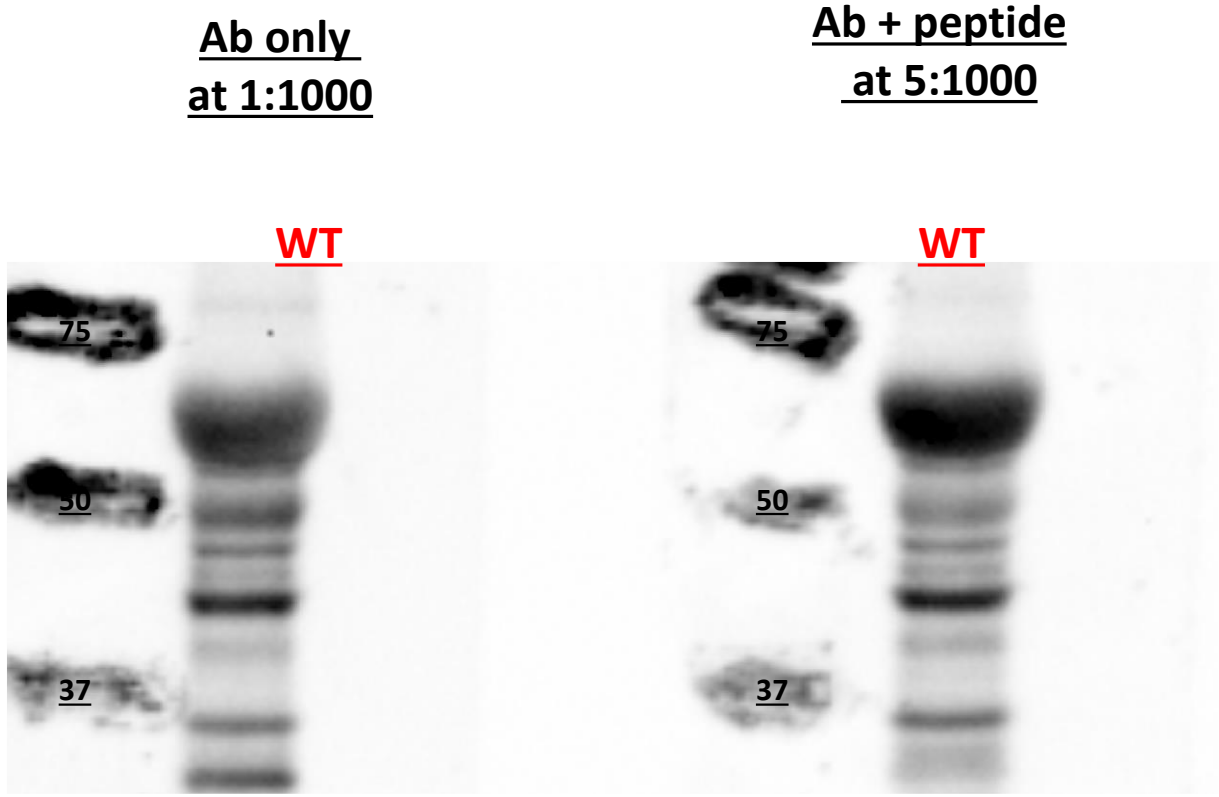


Supplementary Fig. 1B

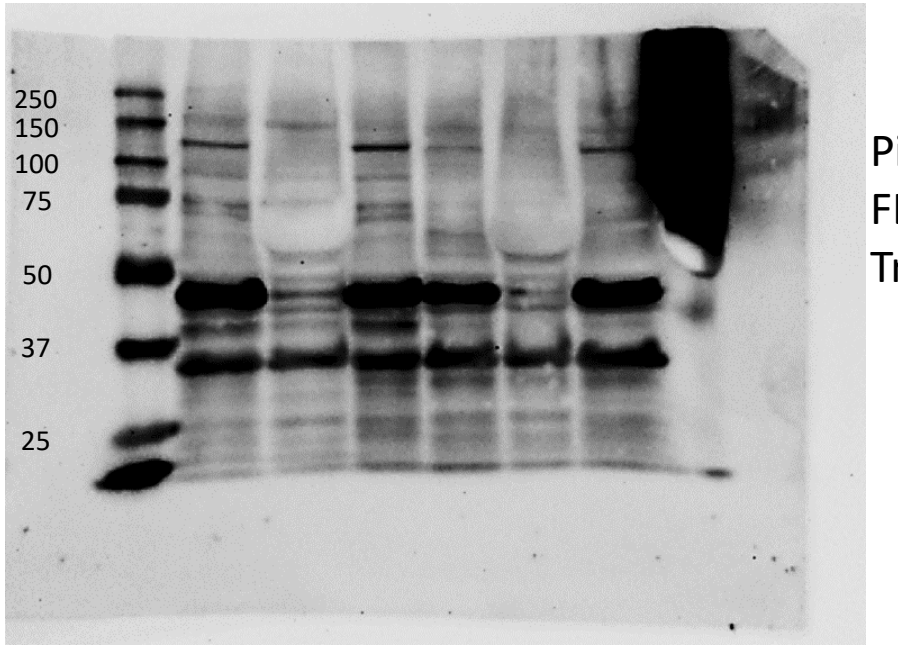


Supplementary Fig. 2b

Exposure 20 seconds

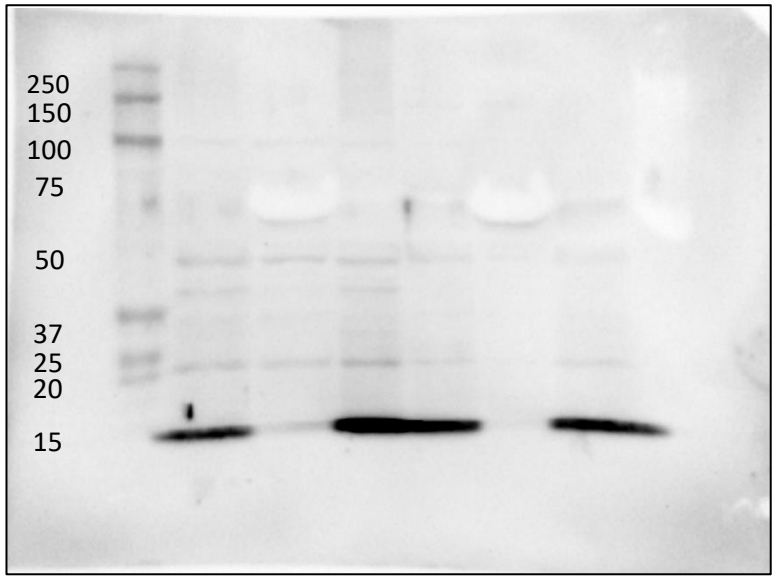


Supplementary Fig. 3



Pink1
FL-63KDa
Truncated-48Kda

B-Tubulin=50kDa

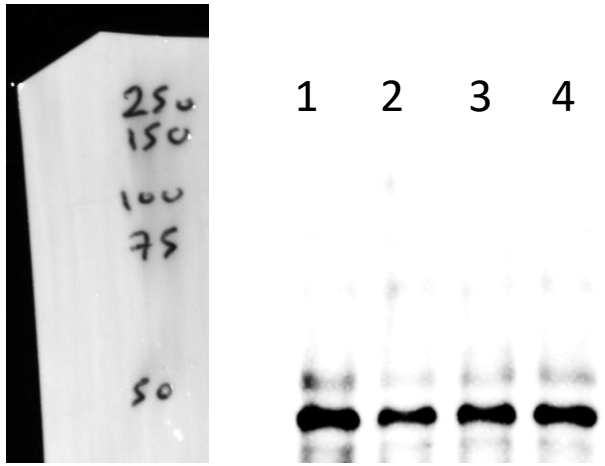


Tom20=15KDa

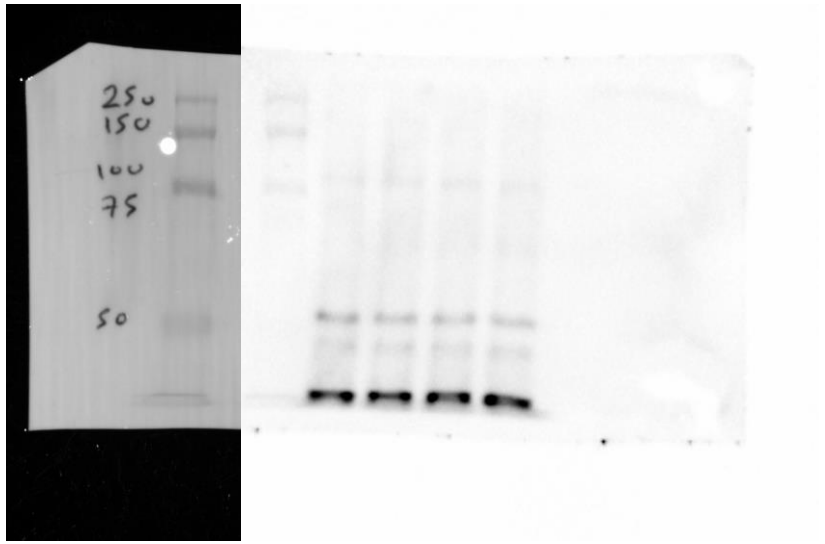
Supplementary Fig. 4

1: DMSO
2: 6h Kinetin
3: 12h Kinetin
4: 24h Kinetin

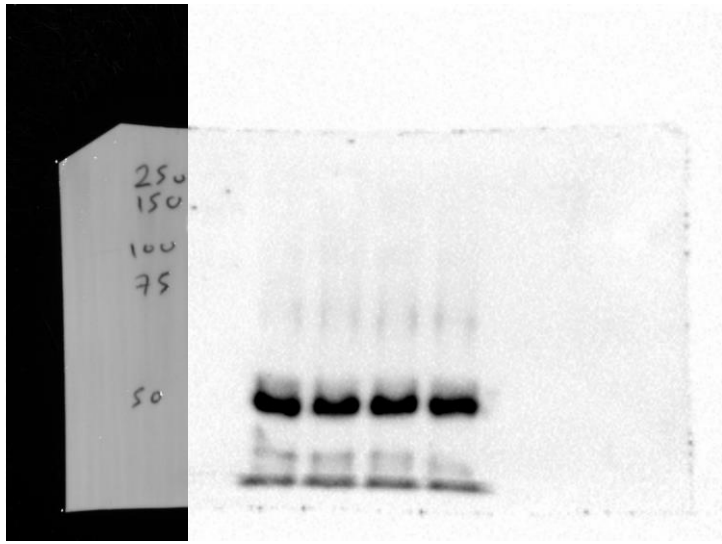
pCREB



CREB



β-Tubulin



Supplementary Fig. 5C